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Garlic Distillate as Botanical Pesticide: A Novel Biorational Control Approach of the Olive Bark Beetle

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ABSTRACT

The susceptibility of olive bark beetle, *Phloeotribus scarabaeoides* (Bernard), larvae and adults, and its parasitoid, *Cheiropachus quadrum*, to garlic distillate was evaluated in laboratory bioassays. The main components of garlic extract were: diallyl-disulfide, diallyl-trisulfide, methyl-allyl-trisulfide, and vinyl-dithiin (1,2-dithiins and 1,3-dithiins) molecules. The highest lethal concentration (LC_{100}) value of garlic distillate was estimated at 3.45 mg/L for *P. scarabaeoides* larvae and at 4.41 mg/L for adult olive bark beetles. The highest mortality rates, reaching 100% for both *P. scarabaeoides* larvae and adults, were obtained following garlic distillate (hydrolat) application at a dose of 8.19 mg/L, whereas a dose of either 0.16 or 0.49 mg/L of garlic distillate resulted in the lowest mortality rates (<40%) for both *P. scarabaeoides* life stages. The mortality rate of adults caused by the artificial blend (full mixture) was significantly lower than that caused by garlic distillate at 7, 14 and 21 days after treatment, while there were no significant differences between both treatments for mortality rates of olive bark beetle larvae. Neither the garlic distillate nor the garlic artificial blend negatively affected the emergence of the parasitoid *C. quadrum* from treated pupae or significantly affected its survival. Hence, garlic distillate acting as a biopesticide, could be developed and applied as a future, eco-friendly promising option for controlling *P. scarabaeoides* occurring in olive orchards.

Keywords: Phloeotribus scarabaeoides; *Cheiropachus quadrum*; garlic distillate; artificial blend; toxicity; olive integrated pest management.

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INTRODUCTION

The olive bark beetle, *Phloeotribus scarabaeoides* (Bernard) (Coleoptera: Scolytidae), is an olive pest of high economic importance in most parts of the Mediterranean area and some parts of Asia (Lozano, Kidd, & Campos, 1996; Lozano, Benazoun, Kidd, & Campos, 1999; Haber & Mifsud, 2007; Fadl, Ibrahim, Afify, & Ahmed, 2010; Deghiche-Diab, Deghiche, & Belhamra, 2021). *Phloeotribus scarabaeoides* attacks can cause severe damage and important economic losses in olive orchards worldwide (Gonzalez & Campos, 1994; Lozano et al, 1996). Research studies have been carried out to identify, develop, and implement the most effective pest management strategies to curb the spread of this pest in olive orchards, aiming to reduce the significant damage and economic losses.

Synthetic chemicals, such as pyrethroid insecticides are commonly used to control *P. scarabaeoides* (Rodriguez, Pena, Sanchez, & Campos, 2003; Ruano, Campos, Sanchez-Raya, & Pena, 2008; Ruano, Campos, Sanchez-Raya, & Pena, 2009), but their repeated application can harm beneficial arthropods and cause significant environmental and human health issues, potentially undermining sustainability objectives (Desneux, Decourtye, & Delpuech, 2007; Rani et al., 2021). Additionally, the overuse and intensive application of broad-spectrum insecticides has caused an increase in the occurrence of resistance to a number of chemical active substances in crop pests (Georghiou, 2013; Bass, Denholm, Williamson, & Nauen, 2015; Hawkins, Bass, Dixon, & Neve, 2019), which mandates the scientific community to evaluate novel eco-friendly and effective alternatives for preventing and/or limiting serious crop damage caused by insects.

In this context, using plant extracts with insecticidal properties could offer a promising, environmentally friendly, and sustainable approach to pest control (Pavela & Benelli, 2016; Isman, 2020; Mansour & Biondi, 2021). Plants produce chemical substances that act as defensive mechanisms to reduce feeding injury by phytophagous organisms (Isman 2020; Lopes Souto et al, 2021). Recent studies have shown the effectiveness of plant extracts against pests and diseases when attacking plants. These natural chemical compounds have various modes of action such as contact, fumigant, anti-feeding effects and cuticle disruption (Isman, 2020; Lopes Souto et al, 2021). Garlic (*Allium sativum* L.), belonging to the family Liliaceae, has been used worldwide for its numerous culinary and medicinal benefits (Rivlin, 2001). It has been extensively tested against human diseases (Sahidur, Islam, & Jahurul, 2023), whereas studies examining its effects on plant pathogens are far less common. One of the earliest studies of using garlic for controlling plant pathogens was performed by Russel & Mussa (1977) who found that garlic extract inhibited the in-vitro growth of *Fusarium solani* f. sp. *phaseoli*, while seed treatment resulted in adequate in-vivo control of foot rot of *Phaseolus vulgaris* cv. *Seafarer*. Since then, extensive research has been performed on the possible use of garlic extracts as alternative to synthetic chemical pesticides. The antifungal activity of garlic essential oil, tested at various concentrations, was demonstrated in vitro, showing strong inhibitory effects against *Fusarium oxysporum* (ATCC 11850), especially at lower concentrations (Benkeblia, 2004). Additionally, antifungal activity was observed with aqueous extracts from 28 garlic cultivars against four fungal species: *Botrytis cinerea*, *F. oxysporum*, *Phytophthora capsici*, and *Verticillium dahliae* (Hayat et al., 2016). Furthermore, in India, garlic bulb extract also demonstrated promising efficacy against mustard blight disease caused by *Alternaria* spp. (Meena et al, 2004; Sharma, Ahir, Yadav, Sharma, & Ghasolia, 2021). There have been several reports of the antibacterial activity of garlic extracts against bacteria. Indeed, in vitro assay with aqueous garlic extract generated a significant efficacy against seed-borne infections that cause bacterial spot disease of tomato (Karabuyuk & Aysan, 2018). Similarly, garlic extracts exhibited interesting activity against *Pseudomonas syringae* pv. tomato, *Xanthomonas vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis*, causal agents of bacterial speck, bacterial spot and bacterial canker, respectively (Balestra, Heydari, Ceccarelli, & Ovidi, 2009). In addition to antimicrobial activity, garlic crude and aqueous extract exhibited promising pesticidal activities against several plant pests including the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) (Attia et al, 2012, 2013), the armyworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), the leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Meriga, Mopuri, & Krishna, 2012; Hamada, Awad, El-Hefny, & Moustafa, 2018), the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Ali et al, 2014), and more recently, the root-knot nematode *Meloidogyne* sp. and the termite *Nasutitermes* sp. (Khairan et al, 2021).

The purpose of the present study was to evaluate the effects of garlic (*A. sativum*) extract applied as either garlic distillate (hydrolat), artificial blend (full mixture) or individual garlic extract component on the olive pest *P. scarabaeoides* and its parasitoid *Cheiropachus quadrum* (Fabricius) (Hymenoptera: Pteromalidae). The main aim was to develop a new biopesticide based on major compounds extracted from garlic for controlling *P. scarabaeoides* occurring in Mediterranean olive orchards, as a sustainable alternative to applying broad-spectrum, hazardous chemical pesticides.

MATERIALS AND METHODS

Chemical analysis and extraction

The garlic extract steam distillate was obtained from 10 kg of garlic cloves by hydro-distillation for 3 h using a Clevenger-type apparatus and then was analyzed by high-performance liquid chromatography coupled with photodiode-array detection (HPLC-DAD) in the laboratory of BioXtract S.A. (Gembloux Agro-Bio Tech, University of Liège, Belgium).

HPLC-DAD analysis

High-performance liquid chromatography (HPLC) separation was performed on a Waters HPLC-DAD system (Alliance 2695, PDA 2996) with an Inertsil ODS 3µ* 3mm* 250 mm column. The following mobile phase and gradient program were used for compound elution: initial conditions were 75% mobile phase A (water) and 25% mobile phase B (acetonitrile) for 1 min at a flow rate of 0.4 mL/min; a linear gradient to 90% phase A was developed over 59 min, then returned to initial conditions until 65 min.

To stabilize the chromatography system, the column was maintained at 30 °C. A new sample injection was possible after 80 min. A Waters 2996 Photodiode array detector was set to a wavelength range of 190-300 nm for detection of garlic compounds, with a sampling rate of 2 Hz. Quantification of garlic extract components was carried out at 210 nm, against calibration using isolated standards furnished by BioXtract S.A., Belgium.

Effect of garlic distillate on *Phloeotribus scarabaeoides* adults and larvae

All the branches were taken from trees in the same part of the olive grove (var. Chetoui) in the regions of Nabeul and Tunis (northeastern Tunisia). Maximum diameter of the main axis of the branches was 4-6 cm. The density of beetle infestation ranged from 100 to 150 overwintering galleries per branch. The experiment consisted of spraying 5 non-infested trunks with each of the following concentrations of garlic distillate: 0.16; 0.49; 0.81; 1.14; 1.47; 2.45; 3.27 and 8.19 mg/L. For the whole experiment, a total of 40 trunks were treated, with five replicates per treatment (concentration). Then 25 adults were randomly collected from infested branches and were maintained in a plastic box with a capacity of 1 L of air in a climate room at 30 °C, 50-60 % RH and 16:8 (L:D) at the National Agronomic Institute of Tunisia, without any contact with insecticides before the experiments. The strain was reared on new branches. Only young adults were chosen for the bioassays. Mortality of *P. scarabaeoides* larvae and adults caused by the garlic distillate, full mixture, and individual constituents was evaluated at 1, 3, 7, 14 and 21 days after treatment, with five replicates performed per treatment.

The toxicity experiments were conducted with individual constituents of the garlic distillate at their natural proportions (HPLC; Fig. 1) in the distillate and in amounts present in the minimum of 100% of the lethal dose of garlic distillate (4.41 mg/L). Pure compounds were provided by BioXtract S.A. Purities of the compounds varied from 95 to 99%. To identify the relative toxicity of each constituent to the toxicity of the distillate, we tested each of these constituents individually. We then tested a blend of all major constituents (full mixture), called artificial blend.

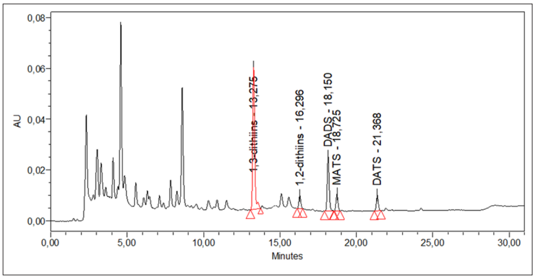

Figure 1. LC-DAD spectra of major organosulfur compounds isolated from garlic (DADS= Diallyl-disulfide, MATS= Methyl-allyl-trisulfide, DATS= Diallyl-trisulfide).

Effect on the emergence and survival of the parasitoid *Cheiropachus quadrum*

Twenty-five *P. scarabaeoides* parasitized individuals (pupae) were taken randomly from each experimental brunch. After treating the pupae with garlic distillate, using a hand sprayer by direct contact from a height of 1 meter, the percentage of emergence of the parasitoid *C. quadrum* was then recorded. Mortality of the parasitoid *C. quadrum* caused by the distillate, full mixture, and individual constituents was assessed when all of these treatments were applied at levels equivalent to those found in the 100% lethal concentration of the garlic distillate (LC_{100}=4.41 mg/L). Five replicates were performed per treatment with 25 parasitoid individuals per replicate.

Data analysis

The LC_{50}, LC_{90} and LC_{100} values were determined by probit analysis using the StatPlus program v. 2009, AnalystSoft Inc. Data was analyzed using one-way analysis of variance (ANOVA), and Tukey's post-hoc test was used to compare treatment means using GraphPad Prism v.8.01 for Windows (GraphPad Software, San Diego, California, USA (http://www.graphPad.com)). All statistical analyses were applied under two-tailed hypotheses and the significance level *P* was set at 0.05. Data corresponding to percentages of efficacy (mortality) and emergence were corrected using the Abbott's formula (Abbott, 1925).

RESULTS

HPLC-DAD analysis of garlic distillate

The components of the *A. sativum* plant extract, identified by HPLC-DAD analysis, are mainly represented by diallyl-disulfide (18.15%), diallyl-trisulfide (21.36%), methyl-allyl-trisulfide (18.72%), in addition to 1,2-dithins (16.29%) and 1,3 dithiins (13.27 %) (Fig. 1).

Toxicity of garlic distillate toward *P. scarabaeoides* larvae and adults

Lethal concentration values (LC_{50}, LC_{90} and LC_{100}) linked to contact toxicity of garlic distillate were lower for *P. scarabaeoides* adults compared to those obtained for the larvae, while the highest lethal concentration (LC_{100} (mg/L)) value was estimated at 3.45 for *P. scarabaeoides* larvae and at 4.41 for adults (Table 1). Based on contact toxicity bioassays, there was a highly significant effect of all garlic distillate doses in inducing mortality of adult beetles at 24 h after treatment ($F_{(8,36)}$=110.8; P< 0.0001) compared to the untreated control. The highest mortality rates, reaching 100% for *P. scarabaeoides* adults, were obtained following garlic distillate (hydrolat) application at a dose of 8.19 mg/L, whereas the lowest mortality rates, being lower than 25%, were obtained after applying the distillate at a dose of either 0.16 or 0.49 mg/L (Table 2).

Table 1. Lethal concentration values (LC50, LC90 and LC100) for garlic distillate (contact toxicity) against *Phloeotribus scarabaeiodes* adults and larvae, estimated using probit analysis.

Concentrations (mg/L)	LC50 (mg/L)	LC90 (mg/L)	LC100 (mg/L)
Adults	1.73	3.98	4.41
Larvae	0.97	3.04	3.45

Similarly to adults, there was a significant difference between the untreated control and garlic treatments on mortality of larvae. Indeed, at 24 h after treatment, garlic essential oil doses significantly affected survival of *P. scarabaeoides* larvae ($F_{(8,36)}$=300.3; P<0.0001). Results showed that the highest mortality rates (100%) of *P. scarabaeoides* larvae were obtained following garlic distillate application at a dose of either 1.47, 2.45, 3.27 or 8.19 mg/L (Table 2). Therefore, garlic distillate applied at a dose of 8.19 mg/L could provide a promising control potential against both *P. scarabaeoides* larvae and adults in olive orchards.

Table 2. Contact toxicity (24 h after treatment) of different concentrations of garlic distillate toward *Phloeotribus scarabaeiodes* larvae and adults.

Concentration (mg/L)	0.16 mg/L	0.49 mg/L	0.81 mg/L	1.14 mg/L	1.47 mg/L	2.45 mg/L	3.27 mg/L	8.19 mg/L
Adults	8.±0.8a	20±3.34a	44±2.33b	54±4.66c	57±6c	78±3.2d	80.8±3.2d	100±0e
Larvae	12.80±2.55a	36±5.08b	65±1.93c	87±3.61d	100±0e	100±0e	100±0e	100±0e

Means ± SEM of percent mortality followed by the same letter within each row are not significantly different (Tukey's test, P<0.05).

Toxicity of garlic distillate, artificial blend, and individual constituents toward *P. scarabaeoides* larvae and adults

There was a significant effect of treatments regarding the lethal toxicity toward *P. scarabaeoides* larvae ($F_{(6, 140)}$=571.4; P<0.0001). All constituents (MATS, DATS, DADS and Dithiins) were highly toxic to larvae compared to the control, but except for the constituent DADS, all other compounds, including the artificial blend, did not induce significantly different toxicity compared to the garlic distillate (Table 3). Overall, all treatments of garlic distillate, artificial blend and individual constituents significantly affected the survival of *P. scarabaeoides* larvae (Table 3) and adults (Table 4) until 21 days after treatments, compared to the control. However, the mortality rate of adults caused by the full mixture (a blend of all 4 major constituents) was significantly lower (P<0.0001) than that caused by garlic distillate at 7, 14 and 21 days after treatment (Table 4).

Table 3. Mortality caused by the distillate, artificial blend, and individual constituents to *Phloeotribus scarabaeiodes* larvae when applied at levels equivalent to those found in the 100% lethal concentration of the distillate (LC100=3.45 mg/L). Values are means ±SE of five replicates with 25 larvae per replicate.

Treatments	1 Dat (%)	3 Dat (%)	7 Dat (%)	14 Dat (%)	21 Dat (%)
Garlic distillate	32±4.35a	40.2±4.77a	45.6±4.96a	49.4±5.6a	59.8±1.02a
Artificial blend	16.4±3.2a	20.2±4.2a	23.2±4.45a	29.8±3.5a	34.8±3.2a
MATS	43.6±6.42a	47.6±7.0a	50±6.98a	51±6.5a	51±6.5a
1,2- dithiins	41±2.17a	43±2.3a	45.2±1.93a	48.2±0.97a	51.6±1.4a
DADS	67±5.02b	72.8±4.40a	75.8±5.02b	75.8±5.02b	76±4.89b
1,3- dithiins	11.4±2.44a	15±3.45b	15.2±3.57a	15.4±3.43a	15.8±3.43c
DATS	0c	0c	0c	0c	0d
Control	0c	0c	0c	0c	0d

Means ± SEM of percent mortality followed by the same letter within each column are not significantly different (Tukey's test, P<0.05).
Dat: days after treatment; %: Abbott's percentage of mortality.

Table 4. Mortality caused by the distillate, artificial blend, and individual constituents to *Phloeotribus scarabaeiodes* adults when applied at levels equivalent to those found in the 100% lethal concentration of the distillate (LC100=4.41 mg/L). Values are mean ±SE of five replicates with 25 adults per replicate.

Treatments	1 Dat (%)	3 Dat (%)	7 Dat (%)	14 Dat (%)	21 Dat (%)
Garlic distillate	40.8±2.46a	60.4±3.54a	82.4±4.8a	100±0a	100±0a
Artificial blend	48±5.32a	64.2±6.58a	71±4.67ab	76.6±3.87b	81.6±2.87b
MATS	91±2.3b	92±2.02b	94±2.26b	95.8±2.01a	93.8±2.01a
1,2-dithiins	97.4±1.94c	98.4±1.03c	99.6±0.4c	99.8±0.2a	100a
DADS	86.2±5.4b	88.8±5.4b	93±2.53c	93.4±3.84a	99±0.45a
1,3-dithiins	45±5.88a	55.6±4.43a	64±2.68d	67.8±3.44c	68.8±3.79c
DATS	0d	0d	0e	0d	0d
Control	0d	0d	0e	0d	0d

Means ± SEM of percent mortality followed by the same letter within each column are not significantly different (Tukey's test, P<0.05). Dat: days after treatment; %: Abbott's percentage of mortality.

Effect on the emergence and survival of the parasitoid *Cheiropachus quadrum*

The percentage of parasitoid emergence obtained in garlic distillate, artificial blend, MATS, DADS and DATS treatments did not differ significantly from that obtained in the untreated control ($F_{(7, 32)}$=11.90; *P*<0.0001), while either garlic distillate individual constituent, 1,2- dithiins or 1,3- dithiins significantly affected the emergence of this parasitoid (Fig. 2). Additionally, garlic distillate, when applied at levels equivalent to those found in the LC_{100}=4.41 mg/L corresponding to *P. scarabaeoides* adults, slightly affected the survival of *C. quadrum* adult parasitoids, with 22% mortality at 14 days after treatment and less than 15% mortality at either 1, 3 or 7 days after treatment, while there were significant differences (*P*<0.0001) between the untreated control and the other treatments except for either 1,3-dithiins or DATS (Table 5). These findings clearly indicate that garlic distillate, being harmful toward *P. scarabaeoides* adults, could not generate harmful side effects toward *C. quadrum* pupae and adults occurring in olive orchards, and its future application as biopesticide could be recommended instead of using the artificial blend or individual garlic extract constituents.

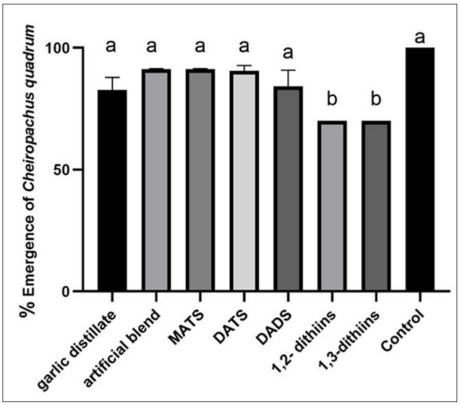

Figure 2. Percentage of emergence of the parasitoid *C. quadrum* at 10 days after garlic distillate treatment, full mixture, and individual constituents when applied at levels equivalent to those found in the 100% lethal concentration of the distillate (LC100=4.41 mg/L). Values are means ± SE of five replicates with 25 nymphs per replicate. Treatments with different letters are significantly different from each other (Tukey's test).

Table 5. Mortality of the parasitoid *C. quadrum* caused by the garlic distillate, artificial blend, and individual constituents when applied at levels equivalent to those found in the 100% lethal concentration of the distillate (LC100=4.41 mg/L). Values are means ±SE of five replicates with 25 nymphs per replicate.

Treatments	1 Dat (%)	3 Dat (%)	7 Dat (%)	14 Dat (%)
Garlic distillate	8±2.35a	11.6±5.86a	14.6±5.98a	22.6±11.52a
Artificial blend	5.4±5.55a	9.2±6.06a	13.2±5.81a	16.4±3.91a
MATS	4.2±4.92a	5.4±5.9b	6±6.36b	12±3.39a
1,2-dithiins	7.4±5.18a	11.6±3.36a	15.6±4.28a	19.4±5.37a
DADS	2±4.47a	6.4±5.41b	15.8±8.61a	20±9.7a
1,3-dithiins	0b	0c	0c	0b
DATS	0b	0c	0c	0b
Control	0b	0c	0c	0b

Means ± SEM of percent mortality followed by the same letter within each column are not significantly different (Tukey's test). Dat: days after treatment; %: Abbott's percentage of mortality.

DISCUSSION

This study presents the first qualitative evidence of the varying efficacy of garlic distillate in controlling the olive bark beetle *P. scarabaeoides*. The results showed that garlic distillate is toxic to the adult beetles, with the calculated lethal concentrations (LC) at 50%, 90%, and 100% mortality being 1.73, 3.98, and 4.41 mg/L, respectively. Four major components of garlic (*A. sativum*) were identified: MATS, DATS, DADS, and dithiins (1,2-dithiins and 1,3-dithiins). At a concentration of 8.19 mg/L, garlic distillate achieved 100% mortality in both larvae and adults of *P. scarabaeoides*. In contrast, lower doses (0.16 and 0.49 mg/L) resulted in mortality rates not exceeding 40%. Additionally, the study demonstrated that the garlic distillate caused significantly higher mortality in larvae compared to an artificial blend, particularly at 14 and 21 days post-treatment, while there was no significant difference for adult mortality between the two treatments. This highlights the effectiveness of garlic distillate in pest management for *P. scarabaeoides*, especially at higher concentrations.

Previous research has shown that garlic essential oil possesses insecticidal properties against various pest species. For example, wild garlic (*Allium ursinum*) has been proven to reduce the colonization of olive trees by the weevil *Hylesinus fraxini* (Panzer) (Coleoptera: Curculionidae) (Friedrich, Kohlenbrenner, & Schneider, 2020; Helbig & Heber, 2020). Furthermore, garlic extracts have also been shown to be lethal to the two-spotted spider mite *T. urticae*, with mortality observed at LC_{50} = 7.49 mg/L and LC_{90} = 13.5 mg/L. In contrast, lower concentrations (0.36 and 0.74 mg/L) reduced pest fecundity (Attia et al, 2012). Gahukar (2018) reported that farmers use garlic sprays in *Aloe vera* (Liliaceae) plantations as a natural pest control method to avoid the toxic residues from chemical pesticides, which could harm human health and the environment. Since chemical pesticides are costly, alternatives like neem oil, kaolin clay, and garlic have been suggested by Johnson, Ruiz-Diaz, Manoukis, & Rodrigues (2020) for short-term control of scolytid insects, including the olive bark beetle *P. scarabaeoides* and the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae). Ebadollahi & Sendi (2015) also highlighted garlic essential oil as an effective, eco-friendly option for controlling the red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae).

Further studies revealed that garlic oil has neurotoxic effects on pest's nervous system. For instance, Boate & Abalis (2020) found that garlic oil caused altered movement, paralysis, and muscle contractions in pests like aphids, beetles, spider mites, whiteflies, and caterpillars. In this way, Shahriari, Sahebzadeh, Sarabandiand, & Zibaee (2016) showed that *Sitophilus granaries* (L.) (Coleoptera: Curculionidae) adult mortality increased with higher concentrations of essential oils from garlic, clove, anise, matricaria, and celery. Additionally, Soe, Ngampongsai, & Sittichaya (2020) reported that high concentrations of medicinal plant oils, especially clove oil, were highly effective against the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and the pulse beetle *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae).

Although there were no significant differences between garlic distillate and artificial blend on mortality of larvae at 7, 14 and 21 days after treatment, statistical analyses revealed a significantly higher mortality rates of adult beetles in the garlic distillate treatment compared to the artificial blend treatment. Interestingly, neither the garlic distillate nor the garlic artificial blend negatively affected the emergence of the parasitoid *C. quadrum* from treated pupae, whereas either garlic distillate individual constituent 1,2-dithiins or 1,3-dithiins significantly affected the emergence of this parasitoid. These findings could suggest a possible future use of garlic distillate instead of applying the garlic artificial blend or garlic individual constituents against this pest in olive orchards.

Wild garlic *A. ursinum* contains a wide range of volatile compounds like the most dominant in *Allium* spp. (e.g., is a major constituent of the essential oil) "diallyl disulfide" (Helbig & Heber, 2020; Soe et al, 2020; Wu et al, 2020), which is used in agriculture as a biopesticide with a repellant effect against pests such as *H. fraxini* (Helbig & Heber, 2020). This molecule prevents oviposition and provides a behavioral barrier to adult insects (Soe et al, 2020). In a study conducted by Chekki, Snoussi, Hamrouni, & Bouzouita (2014), two major molecules were distinguished in Tunisian garlic essential oil: Diallyl disulfide DADS (44.6%), and Allyl methyl trisulphide (11.8%). Four other components were also identified by the same authors. These are diallyl sulfide DAS (4.1%), Allyl mehyl disulfide (6.5%), 3-vinyl-1,2-dithiin (4.04%) and 2-vinyl-1,3-dithiin (1.2%) (Chekki et al, 2014). However, other analyses showed a difference in the percentage of these molecules. For example, Dially disulfide occupies 10.8% of the garlic extract composition (Attia et al, 2012). This variation can be explained by many factors related to the extraction method (Satyal, Craft, Dosoky, & Setzer, 2017) as well as to the degradation of the Allicin into secondary sulfide products (Brodinitz, Pascal, & Derslice, 1971; Block, 1985). In contrast to these variations, all the studies come around the fact that *A. sativum* presents the highest sulfur concentrations compared to other vegetables like broccoli and onion (Virtaen, 1965). Toxicity analysis showed that these organosulfur components are the reason behind the mortality of mites (Singh, Prithiyirai, Sarma, Singhand, & Ray, 2001; Prischamann, James, Wright, Teneyck, & Snyder, 2005). They have also been proven to have lethal and repellent effects on other pests such as *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), when the components were applied onto the larval, pupal and adult stages. Although diallyl disulfide showed the highest level of toxicity compared to diallyl sulfide, both compounds were considered as promising alternative control solutions for *S. zeamais* and *T. castaneum* (Ho, Koh, Ma, Huang, & Sim, 1996; Huang, Chen, & Ho, 2000). A study conducted by Soe et al. (2020) showed that diallyl disulfide was the most toxic compound against *T. castaneum* fourth larval instar compared to Thymol, α-Pinene and Trans-Anethole substances. Diallyl trisulfide (DAT) is the main bioactive organosulfur content of garlic essential oil, and possesses an insecticidal property against *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). Shah et al. (2020) assumed that the treatment with DAT leads to a substantial effect on the chitin synthase A gene of *S. cerealella* and induces morphological and physiological disorders, which decreased the number of eggs laid by females of this insect species.

Based on the findings of this study, we propose that garlic distillate has the potential to be developed as a new botanical pesticide. This would align with eco-friendly integrated pest management programs for olive groves, supporting sustainable biodiversity and vital ecosystem services. To optimize and ensure the sustainable use of garlic plant extracts in future pest management strategies in Mediterranean olive groves, it is crucial to focus on improving the efficiency of indigenous garlic extraction methods. Additionally, exploring practical applications of these extracts in field treatments would be of significant value for enhancing pest control effectiveness while maintaining environmental balance. In this context, a better understanding of the bio-ecology of olive bark beetle in Tunisia would be of great importance, especially considering that larvae of this insect are always present in hidden locations of the tree, which should be considered when developing and implementing pest management programs using garlic extract treatments.

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